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Initial Adhesion of Methicillin-Sensitive and Methicillin-Resistant *Staphylococcus aureus* Strains to Untreated and Electropolished Surgical Steel Drill Bits

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Abstract

Electropolishing of stainless steel has been thoroughly investigated as a prophylactic measure to prevent bacterial colonization of orthopaedic implants and infection. Initial bacterial adhesion onto surgical drill bits as a possible factor for orthopaedic surgical site infections has not yet been documented. The present study investigated the influence of electropolishing on initial staphylococcal adhesion onto AISI 440A stainless steel drill bits. Specifically, one methicillin-susceptible standard laboratory *Staphylococcus aureus* type strain (DSM 20231^T), one methicillin-resistant *S. aureus* reference strain (DSM 46320) and one methicillin-resistant clinical isolate from an infected orthopaedic implant were used. After standard sterilization, drill bits were immersed in the respective bacterial suspension; bacteria adherent to surface were harvested by vortexing the drill bits in phosphate-buffered saline and viable counts of bacteria transferred from the suspension were made (transferred to log10 for further analysis). Electropolishing significantly reduced adhesion of the clinical *S. aureus* strain and the *S. aureus* DSM 20231^T. However, electropolishing significantly increased adhesion of the *S. aureus* DSM 46320. These results show that electropolishing significantly influences initial adhesion of *S. aureus* strains to surgical drill bits and that the nature of this influence depends on the *S. aureus* strain examined. For a general recommendation of electropolishing drill bits and guidelines for their handling during surgery,

further studies with more strains isolated from infected wounds are suggested.

Keywords

drill bits, orthopedic, surgery, *Staphylococcus aureus*, MRSA, infection

Introduction

Today, little is known about the role of drill bits in the complex hierarchy of factors contributing to orthopedic SSIs. This is in stark contrast to extensive research on the influence of composition and surface characteristics of metallic implants used in orthopaedic surgery (Gallo et al., 2014; Rochford et al., 2012; Sullivan et al., 2014; Veerachamy et al., 2014). The authors are not aware of other studies investigating the role of initial bacterial adhesion onto surgical drill bits in this context.

Surgical site infections (SSIs) remain an inevitable risk of any surgical procedure (Andrade et al., 2016; Eugster et al., 2004; Lindeque et al. 2014) despite routine use of aseptic techniques in modern veterinary surgical practices and widespread use of antimicrobials; they are a major limiting factor for long-term success in orthopaedic surgery (Brown et al., 2016; Şen and Kiliç, 2012; Weese, 2008). Within the large variety of pathogens involved in SSIs, *S. aureus* is a lead microbial agent in human and veterinary nosocomial infections and the emergence of methicillin-resistant species (MRSA) has made successful treatment of SSIs more challenging and overall less successful (Andrade et al., 2016; Sasi et al., 2015; Weese and van Duijkeren, 2010).

Many risk factors for SSIs have been identified, one of them is bacterial wound contamination (Eugster et al., 2004; Morley, 2004; Weese, 2008). These bacteria originate from the surgical team, the environment and

the materials and instruments used (Verwilghen and Singh, 2015). Intraoperative pathogen transfer is difficult to control, as surgical sites as well as surgical instruments are commonly contaminated by airborne and endogenous pathogens during surgery. Air ventilation systems reducing airborne bacterial counts are rarely available in veterinary hospitals, and even thus equipped ultraclean surgical theatres in human hospitals are not pathogen free (McHugh et al., 2015; Pada and Perl, 2015).

Electropolishing of surgical steel is a contact-free electrochemical process by which micro-protrusions are removed and a thicker and more uniform protective passive layer is created, resulting in a smoother and more corrosion resistant surface. Electropolishing has been shown to reduce bacterial adhesions on surgical implant materials and food-contact surfaces (Bohinc et al., 2016; Harris et al., 2007). Variables influenced by electropolishing, such as roughness and chemical composition of materials, are critical factors for initial bacterial adhesion (Bos et al., 1999; Costerton et al., 1999; Dunne, 2002; Vickery et al., 2013). Today, orthopaedic implant surfaces are designed to counteract bacterial adhesion while surgical instruments with short-term tissue contact such as drill bits are predominantly designed for repeated use, focussing on e.g. sharpness of cutting edges and corrosion resistance. Repeated use of such surgical instruments necessitates washing, cleaning and disinfection and/or sterilization procedures; however the influence of such procedures on bacterial adhesion is as yet unclear.

The following hypothesis was investigated in the present study: adhesion of methicillin-sensitive and methicillin-resistant *S. aureus* strains is significantly different on untreated and electropolished surgical steel drill bits.

Material and Methods

Figure 1 gives an overview of all experimental steps in chronological order.

Drill bits

For the present study, commercially available untreated and electropolished, unused and used surgical drill bits (DePuy Synthes) were used (Table 1). These drill bits were previously reported on in a study focusing on the effects of drill use, washing and sterilization on their surface characteristics (Henkel et al., 2011). An overview of all experimental steps performed in that earlier study is shown in Figure S2.

Briefly, that study compared two-dimensional (2D) roughness, pores and corrosion of untreated and electropolished, two-fluted surgical steel (AISI 440A) drill bits before and after use for drilling equine cortical bone. Untreated and electropolished drill bits were used for drilling into equine cortical bone, followed by routine washing and sterilization procedures (see paragraph "Washing and Sterilization" below). Surface pores were then determined microscopically and classified according to size. The the number of small pores decreased significantly with use, while

electropolishing significantly increased 2D roughness (Ra) and the number of large pores (Fig. 2) (Henkel et al., 2011).

In the present study the number of pores irrespective of size was used for analysis. A random selection of 45 untreated (UT) and 45 electropolished (EP) drill bits from the 100 used by Henkel et al. were used for the present study resulting in the following experimental groups:

- 9 UT and 9 EP unused drill bits;
- 9 UT and 9 EP drill bits used for 5 drill holes;
- 9 UT and 9 EP drill bits used for 15 drill holes;
- 9 UT and 9 EP drill bits used for 25 drill holes;
- 9 UT and 9 EP drill bits used for 40 drill holes.

Bacteria

Three *S. aureus* strains were used in the present study. One methicillin-susceptible *S. aureus* type strain (*Sa*T; DSM 20231^{T}), one methicillin-resistant *S. aureus* (MRSA) reference strain (*Sa*R; DSM 46320) and a third *S. aureus* strain (*Sa*C) isolated from a chronically infected fracture repair of a foal at the Clinic for Horses (University of Veterinary Medicine Vienna, Austria). Total DNA was extracted from all strains with a slightly modified procedure from that provided by the manufacturer (UltraClean® Microbial DNA Isolation Kit, MoBio Laboratories, Qiagen, USA) as follows: after adding the aqueous lysis solution MD1, the cells were heated at 65 °C for a prolonged step of 45 minutes (Loncaric et al., 2014). All three *S. aureus* strains were subjected to PCR detecting PVL-

gene, SCC*mec*, *spa* typing (Loncaric et al., 2014), *mecA*-PCR (Loncaric et al., 2016) and Multi Locus Sequence Typing (MLST). MLST was carried out by PCR amplification and sequencing of seven housekeeping genes (*arcC*, *aroE*, *glpF*, *gmk*, *pta*, *tpi*, *yqiL*) as previously described (Loncaric et al., 2013). Antimicrobial susceptibility testing to cefoxitin was performed by agar disk diffusion method according to Clinical and Laboratory Standards Institute (CLSI, 2013). All three strains were also screened for the presence of the gene encoding the intercellular adhesion protein A *(icaA)* (Loncaric et al., 2016) associated with biofilm formation, and for genes coding for adhesins collagen *(cna)*, fibronectin *(fnbA)*, surface-located fibrinogen binding proteins (clumping factor A and B, *clfA* and *clfB*) (Van Leeuwen et al., 2005) that encode microbial surface components recognizing adhesive matrix molecules (MSCRAMMs). Further, the strains were tested for the presence of metal resistance determinants via PCR amplification using oligonucleotides for genes conferring resistance to arsenic compounds *(arsA)*, copper *(copB)*, cadmium *(cadD)* and zinc *(czrC)* (Argudín and Butaye, 2016; Cavaco et al., 2011) as well as antiseptic resistance encoding genes (quaternary ammonium compounds, QAC) *qacA* and *smr* (Sidhu et al., 2002) (Table 2).

Blood contamination

Forty-two of the drill bits were subjected to blood staining before bacterial contamination to test whether either surface would retain residual organic material, potentially enhancing bacterial adhesion. Leftovers from

blood samples of healthy horses collected in EDTA vacutainer tubes (Vacuette®, Greiner Bio-One GmbH) for pre-surgical routine laboratory testing were obtained from the Clinic for Horses. The fluted cutter part of each drill bit (50 mm) was immersed in 5 mL of equine blood for 1 minute in 15 mL sterile centrifuge tubes (Sarstedt) before fixation with 75% ethanol (Merck) for 10 seconds, and drill bits were allowed to dry at room temperature. This was repeated three times before these drill bits were returned to the remaining 48 drill bits for washing, sterilization and experiments with bacterial strains.

Washing and Sterilization

All drill bits were washed and autoclaved before they were used for bacteriological experiments. The washing procedure was carried out in an automated washer (G7883CD, Miele) following EN ISO 15883-1 with the Vario TD washing program using a washing agent and neutralizer (neodisher and neodisher N, LaboClean FLA, Dr. Weigert). For sterilization each drill bit was packed separately (Medipeel, Sengewald) and sterilized in a steam autoclave (Getinge Group) with the following parameters: 121 °C, 1.2 bar in chamber and jacket, 2.3 – 2.4 bar steam supply for 55 minutes. Both the washing and the sterilization procedure followed the standard protocol of the Clinic for Horses.

Inoculum preparation, recovery media and inoculation method

All *S. aureus* strains were cultivated on Tryptone Soy Agar (TSA, CARL ROTH GmbH + Co. KG) from frozen stocks for 20 – 24 hours at 37 °C. Tryptic Soy Broth (TSB, BD™ Tryptic Soy Broth, Becton Dickinson GmbH) was used as liquid cultivation medium. After cultivation of bacteria in TSB to late log phase, the medium was centrifuged for 5 minutes at 12,396 RCF in 2 mL tubes. Bacteria pellets were re-suspended into phosphate buffered saline (PBS) and diluted to a turbidity of 0.5 McF. For each drill bit, 5 mL of 0.5 McF bacterial suspension was pipetted into 15 mL tubes (Sarstedt). This volume provided total submersion of the drill bits' cutter part. Drill bits were incubated for 5 minutes at room temperature, then 20 minutes with agitation on an orbital motion shaker with 150 rpm (Model 3019, GFL Gesellschaft für Labortechnik mbH) at 37 °C and then again 5 minutes at room temperature. Drill bits were removed from the tubes under sterile conditions and flushed carefully with PBS to remove non-adherent bacteria from the surface. After flushing, drill bits were transferred into 15 mL tubes containing 5 mL of sterile PBS. After vortexing for 60 seconds to detach adherent bacteria, drill bits were removed from the tubes. In a 2 mL tube, 100 µL of the retained PBS (containing detached bacteria) were suspended in 900 µL of fresh sterile PBS once, twice or three times; dilutions 10^{-1}, 10^{-2} and 10^{-3} were used. From each dilution, 100 µL were streaked onto TSA plates in triplicate and incubated for 20 – 24 hours at 37 °C. This yielded 9 TSA plates per drill bit.

Assessment of bacterial adhesion

Viable counts were performed for each drill bit and median values of two or three plates of the same dilution were used for statistical analyses. The mean value of colony forming unit (CFU) counts of the plates per drill bit was calculated and transformed into log10. Transformed mean CFU data showed normal distribution.

Statistical Analysis

The assumption of normal distribution was tested using Kolmogorov-Smirnov test. The influence of *S. aureus* strain, surface, number of drillings and blood contamination on bacterial adhesion was analyzed using a general linear model. Post hoc comparisons were performed using Bonferroni's alpha correction procedure. Pearson´s correlation coefficients between bacterial adhesion (log10 CFU) and surface characteristics (drilling cycles, total drilling depth, pore numbers, total pore area and 2D roughness) were calculated. A p-value below 5% ($p < 0.05$) was seen as significant. All analyses were performed using SPSS (Version 19.0.0, SPSS Inc.) and MS Excel (Office 2010, Microsoft Corporation).

Results

Data was normally distributed and therefore, parametric tests were used for further analysis. One drill bit was excluded from all statistical analyses, based on the result of CFU counts of this drill bit, which were far below the range of the other drill bits (log10 CFU of the excluded drill bit no. 9 was 3.804; range of all other drills log10 CFU was 4.914 to 6.294).

This was potentially caused by an unidentified handling error during laboratory experiments.

Bacteria

Results of all PCR-based methods (*mecA*-PCR, PCR detecting PVL-gene, SCC*mec*, *spa* typing, MLST) and of the gene screenings (*icaA*, *cna*, *fnbA*, *clfA*, *clfB*, metal-resistance determinants, *qacA*, *smr*) of the *S. aureus* strains to are shown in Table 2.

Blood contamination

Bacterial adhesion was not significantly different between drills that were contaminated with blood and uncontaminated drill bits over all three bacterial strains. Likewise, comparing contaminated and uncontaminated drill bits for each bacterial strain separately showed no significant differences.

Use of drill bits

There was no significant correlation between the drilling depth these drill bits had been used for (indicating their wear and tear) and the bacterial adhesion of the three staphylococcal strains; similarly, there was no significant correlation between the drilling cycles, also a measure for the grade of use of the drill bits, and the bacterial adhesion investigated.

Untreated versus electropolished drill bits

Drill bits were assigned to the three staphylococcal strains with similar surface characteristics in all three groups (Table 1). Total pore area and roughness average were significantly different between untreated and

electropolished dill bits for each staphylococcal strain (Table 1). No significant differences were found for any of the characteristics between the drill bits used for *Sa*T (DSM 20231^T; n=30: 15 UT + 15 EP), *Sa*R (DSM 46320; n=28: 14 UT + 14 EP) and *Sa*C (clinical strain; n=29: 15 UT + 14 EP) (Table 1).

The Figure 3 graph represents bacterial adhesion on untreated and electropolished surfaces. For each of the three *S. aureus* strains, we observed a significant difference of bacterial adhesion between untreated and electropolished drill bits. On electropolished drill bits, SaT and SaC strains showed significantly less adhesion (log10 CFU) than on untreated drill bits (SaT UT 5.431 ± 0.230; SaT EP 5.231 ± 0.121; P = 0.006; SaC UT 5.461 ± 0.175; SaC EP 5.241 ± 0.178; P = 0.002). This effect was reversed in the SaR strain, where significantly more bacteria were harvested from electropolished drill bits (SaR UT 5.532 ± 0.207; SaR EP 5.750 ± 0.220; P = 0.012). On untreated drill bits, there was no significant difference in bacterial adhesion between the three different *S. aureus* strains (Figure 3). There were no significant correlations between pore numbers, total pore area or 2D roughness (Ra) and bacterial adhesion.

Discussion

Based on our results, the proposed hypothesis that adhesion of methicillin-sensitive and methicillin-resistant *S. aureus* strains to surgical

steel drill bits is significantly influenced by different surfaces (untreated and electropolished) is accepted.

Staphylococcus spp. account for up to two thirds of surgical site infections related to orthopedic implants and post arthroplasty (Sugarman, 1986; Trampuz and Widmer, 2006; Walls et al., 2008). Bacteria such as *S. aureus* adapt rapidly to the selective pressure of antibiotics which has resulted in the emergence and spread of MRSA, complicating treatment of these SSIs (Deurenberg and Stobberingh, 2009; Faires et al., 2010; Rich, 2005). For the present study, we selected methicillin-sensitive and -resistant as well as laboratory and wild-type *S. aureus* strains. The isolate *Sa*C from an infected wound in a foal was used as an example of a clinical MRSA isolate. The clonal lineage of this strain was ST398, a common colonizer of animals and humans, also reported to cause clinical infection (Couto et al., 2016). It was complemented by a second strain, *Sa*T (DSM 20231^{T}) from a public collection, which represented a methicillin-sensitive (MSSA) wild-type strain. This standard laboratory strain has previously been reported to have biofilm-forming capacity, an important mechanism for causing disease (Becker et al., 2001). To reflect average durations of orthopaedic surgical procedures, our experimental set-up did not allow sufficient time for a biofilm to develop, but the genetic ability of a strain to do so is of interest for adhesion capabilities and therefore relevant to the research question. The third strain, *Sa*R (DSM 46320) from a public collection was chosen as standard laboratory MRSA reference.

The use of metals and metalloids such as mercury, arsenic, antimony, silver and copper as chemotherapeutics or antimicrobials in medicine steadily declined over recent decades in favor of other antimicrobial agents (Hobman and Crossman, 2014). As the development of new antibiotics has not kept up with the emergence of many multidrug-resistant pathogens (e.g. MRSA), metal(loids) may regain their standing as antimicrobial weapons (Hobman and Crossman, 2014). Against this background, it seems reasonable to investigate links between bacterial adhesion and metal resistance genes. As the staphylococcal strains used in this study were all negative for arsenic compounds, copper, cadmium and zinc/cadmium resistance genes, the results of the presents study may potentially also be valid for steel containing any of the above.

The drill bits used in the present study were commercially available untreated and electropolished standard AISI 440A stainless steel instruments. The stainless steel type AISI 440A is a martensitic composition which is not as corrosion resistant as austenitic steel but extremely hard, making it a good choice for instruments such as drill bits which have to withstand high shear forces (Biehler et al., 2017; Clement et al., 2015). Electropolished drill bits were used because electropolishing of metal surfaces has been shown to influence roughness and thereby bacterial adhesion, which is relevant to the research question (Harris et al., 2007). The electropolished drill bits used in the present study showed increased (2D) roughness of surfaces, a parameter determined by cracks, voids and

protrusions commonly summarized as pores (Henkel et al., 2011). In a recent study, two *S. aureus* strains adhered significantly less to electropolished compared to brushed AISI 304 stainless steel (roughness of 380 nm and 100 nm respectively) and SEM confirmed that bacteria on surfaces with higher roughness grew in multilayers and concentrated in pores and scratches (Bohinc et al., 2016).

Adhesion of bacteria onto metallic surfaces and subsequent biofilm formation depends on properties of the microbial agent itself, characteristics of the surface in question and non-specific interaction forces coming into effect between them (Teughels et al., 2006). Bacterial adhesion has been described as a two-stage kinetic binding model, and it is the first interaction stage between the bacterial cell surface and the material surface that the present study focused on (van Loosdrecht et al., 1989). Initial adhesion of bacteria occurs rapidly and is easily reversible, thus we only allowed a relatively short time for adhesion and vortexing was chosen to remove adherent bacteria (van Loosdrecht et al., 1989). In the present study, all three bacterial strains adhered differently onto untreated and electropolished surfaces of drill bits. As adhesion properties are determined by the genetic profile of a bacterial strain, clinical isolates are most important to consider when examining the relevance of certain properties of a particular strain for surgical site infections (SSIs). Bacterial strains adapt their genetic profiles and develop new resistance phenotypes through exposure to and interaction with hosts and their environment. Also, biofilm-producing bacteria have the

ability to detect surfaces and express specific genes leading to synthesis of extracellular matrix after initial attachment (Costerton et al., 1999). Methicillin-resistant *S. aureus* strains with biofilm-producing capability are more adherent than others and evade host defense and antimicrobial therapy within their self-produced biofilm matrix (Harris et al., 2007). In the present study, screening for genes encoding biofilm-producing capabilities was not performed. Potentially, the environment in which SaR had been cultivated in may account for the distinct adhesion pattern compared to SaT and the clinical isolate SaC. Also, the possibility of incomplete and or unequal detachment and recovery of adhered bacteria of the different strains from the drill bits cannot be completely ruled out. Direct staining methods, 3D culture media or detachment through ultrasound might have provided more comprehensive results, but these would not have represented the clinical situation as closely.

Drill bits are exposed to proteinaceous organic material such as blood and tissue during surgery and are often left to dry in the surgical theatre between drillings of a sequence of holes. Alcohol is frequently used for decontamination of surgical instruments before cleaning and sterilization which could further complicate removal of proteinaceous residues (Prior et al., 2004). The present study examined the influence of this on the adhesion of three *S. aureus* strains to untreated and electropolished stainless steel drill bits. Preconditioning layers of adsorbed organic material on stainless steel surfaces and residual infectious proteinaceous contamination of surgical

instruments can promote adhesion of pathogenic microbial agents such as *S. aureus* (Banerjee et al., 2010; Barnes et al., 1999; Lipscomb et al., 2006). Despite commonly applied cleaning and sterilization procedures to various surgical instruments of different size and shape, studies found a substantial percentage of remaining proteinaceous and non-proteinaceous contamination (by visual inspection and microscopic evaluation) after staining (Banerjee et al., 2010; Baxter et al., 2006; Lipscomb et al., 2006; Murdoch et al., 2006; Smith et al., 2011; Smith and Smith, 2012). The washing and sterilization process used for drill bits in this study followed the standard protocol of the clinic, but whether proteinaceous residues were present on the drill bits after the blood coating experiment was not documented. Depending on the composition and thickness of such a protein or proteinaceous layer, it may either inhibit or promote adhesion of microorganisms (Banerjee et al., 2010; Barnes et al., 1999; Lipscomb et al., 2006). Also, longer drying time of substrates such as blood on the instruments' surfaces increases the amount of adsorbed protein onto stainless steel and reduces efficiency of certain cleaning agents (Secker et al., 2011). One study reported a protein load of 7.0 ± 5.9 ng/mm^2 after only 15 minutes drying time, which increased to 148.3 ± 26.5 ng/mm^2 after 60 minutes (Secker et al., 2011). The sigmoidal trend of protein adsorption in relation to drying time between 15 and 60 minutes found in the study by Secker et al. (2011) is in accordance with another study that analyzed drying time of protein onto stainless steel (Lipscomb et al., 2007). This suggests

that contamination can be kept to a minimum by preventing instruments from drying for > 15 minutes and maintaining moist conditions post-contamination significantly reduces protein adsorption which subsequently facilitates successful decontamination of surgical instruments (Secker et al., 2011). In contrast to what has been reported previously (Banerjee et al., 2010; Barnes et al., 1999; Lipscomb et al., 2006), we found no difference of bacterial adhesion on blood adsorbed untreated or electropolished drill bits compared to the drill bits without blood contamination. Future experiments should include prior documentation of presence of organic residues, e.g. by microscopic examination and potentially also use other organic material that drill bits are in contact with during surgery such as fat and muscle tissue.

Conclusions

The present study showed that electropolishing significantly influences initial adhesion of *S. aureus* strains to surgical drill bits. For a general recommendation of electropolishing drill bits and guidelines for their handling during surgery, further studies with more strains isolated from infected wounds are suggested.

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Figure Captions

Fig 1. Experimental steps in chronological order. UT: untreated; EP: electropolished.

Fig 2. Reflected-light microscope photographs of the surface of one drill bit (number 13) before and after electropolishing. Photographs show part of the surface of a drill bit before (left, A) and after (right, B) electropolishing. The investigation area of 920.87 µm2 is outlined with a white square, and pores of different size are colored, with each color assigned to one range of sizes. Magnification 200.

Fig 3. Adhesion of three staphylococcal strains on untreated and electropolished drill bits. Box plots showing the number of bacteria as log10 colony forming units (CFU) of each of the three *S. aureus* strains harvested from the surface of untreated (light grey) or electropolished (dark grey) surgical drill bits. Horizontal lines within the boxes show the median values with upper and lower boundaries indicating the 25^{th}- and 75^{th}-percentile. Whiskers reach the lowest and highest values except for statistical outliers represented as circles below or above the whiskers.

Supporting Information Captions

S1 Figure. Drill bit. Photograph of an untreated drill bit with metric scale.

S2 Figure. Experimental steps of previous study into electropolishing of surgical drill bits (adapted from Henkel et al., 2011). SEM: scanning electron microscopy. EDX: energy-dispersive x-ray spectroscopy.

Table 1. Characteristics of surgical drill bits.

S.aureus Strain	Surface	Drilling Cycles	Drilling Depth [cm]	Number of Pores	Pore Area [μm^2]	2D Roughness average [µm]
*Sa*T (DSM 20231^T)	UT	3,80 (± 2,57)	77.76 (± 68.53)	402.70 (± 64.36)	15.85 (± 7.59)	0.18 (± 0.02)
	EP	3.80 (± 2.57)	78.09 (± 69.15)	345.23 (± 45.87)	17.77 (± 7.43)	0.36 (± 0.05)
*Sa*R (DSM 46320)	UT	3.86 (± 2.66)	62.96 (± 60.86)	470.68 (± 69.41)	15.48 (± 7.06)	0.18 (± 0.02)
	EP	4.00 (± 2.54)	92.54 (± 69.82)	366.18 (± 39.52)	17.63 (± 6.98)	0.34 (± 0.05)
*Sa*C (clinical isolate)	UT	3.80 (± 2.57)	79.41 (± 68.77)	452.57 (± 58.48)	15.97 (± 7.40)	0.18 (± 0.02)
	EP	3.86 (± 2.66)	84.63 (± 67.50)	368.96 (± 66.78)	18.36 (± 6.57)	0.37 (± 0.06)

Characteristics of the untreated (UT) and electropolished (EP) drill bits used for bacterial adhesion testing in the present study. There is no significant difference between any of the characteristics of the drill bits used for the three *S. aureus* strains. Details for each drill bit were sourced from Henkel et al., 2011 (see also supplemental Figure S2). The mean value for each characteristic is presented. One drilling cycle refers to the drilling of 5 holes in equine cortical bone and the drilling depth is the overall depth of equine cortical bone drilled with each drill bit. Investigation area for number of pores and total pore area was 920.87 μm^2.

Table 2. Characteristics of *Staphylococcus aureus* strains.

Staphylococcal strain	Resistance phenotype	SCC*mec*[a] type	*mecA* gene	PVL[b] genes	*spa*[c] type	MLST[d] type
*Sa*T (DSM 20231^T)			-	-	t211	ST8
*Sa*R (DSM 46320)	FOX[e]	III	+	-	t037	ST239
*Sa*C (clinical isolate)	FOX	IVa	+	-	t011	ST398

Staphylococcal strain	Heavy metal resistance genes				QAC resistance genes	
	arsA[f]	*copB*[g]	*cadD*[h]	*czrC*[i]	*qacA/B*[j]	*smr*[j]
*Sa*T (DSM 20231^T)	-	-	-	-	-	-
*Sa*R (DSM 46320)	-	-	-	-	-	-
*Sa*C (clinical isolate)	-	-	-	-	-	-

Staphylococcal strain	Adhesins				
	fnbA[k]	*clfA*[l]	*clfB*[l]	*icaA*[m]	*cna*[n]
*Sa*T (DSM 20231^T)	+	+	+	+	-
*Sa*R (DSM 46320)	+	+	+	+	+
*Sa*C (clinical isolate)	+	+	+	+	-

QAC: quarternary ammonium compounds; [a]staphylococcal cassette chromosome *mec*; [b]Panton-Valentine leukocidin; [c]Protein A encoding gene; [d]multilocus sequence typing; [e]cefoxitin; [f]arsenic compounds resistance gene; [g]copper resistance gene; [h]cadmium resistance gene; [i]zinc/cadmium resistance gene; [j]quaternary ammonium compounds resistance gene families; [k]fibronectin; [l]clumping factor (surface-located fibrinogen binding protein) A and B; [m]intercellular gene cluster adhesion operon; [n]collagen adhesin gene.

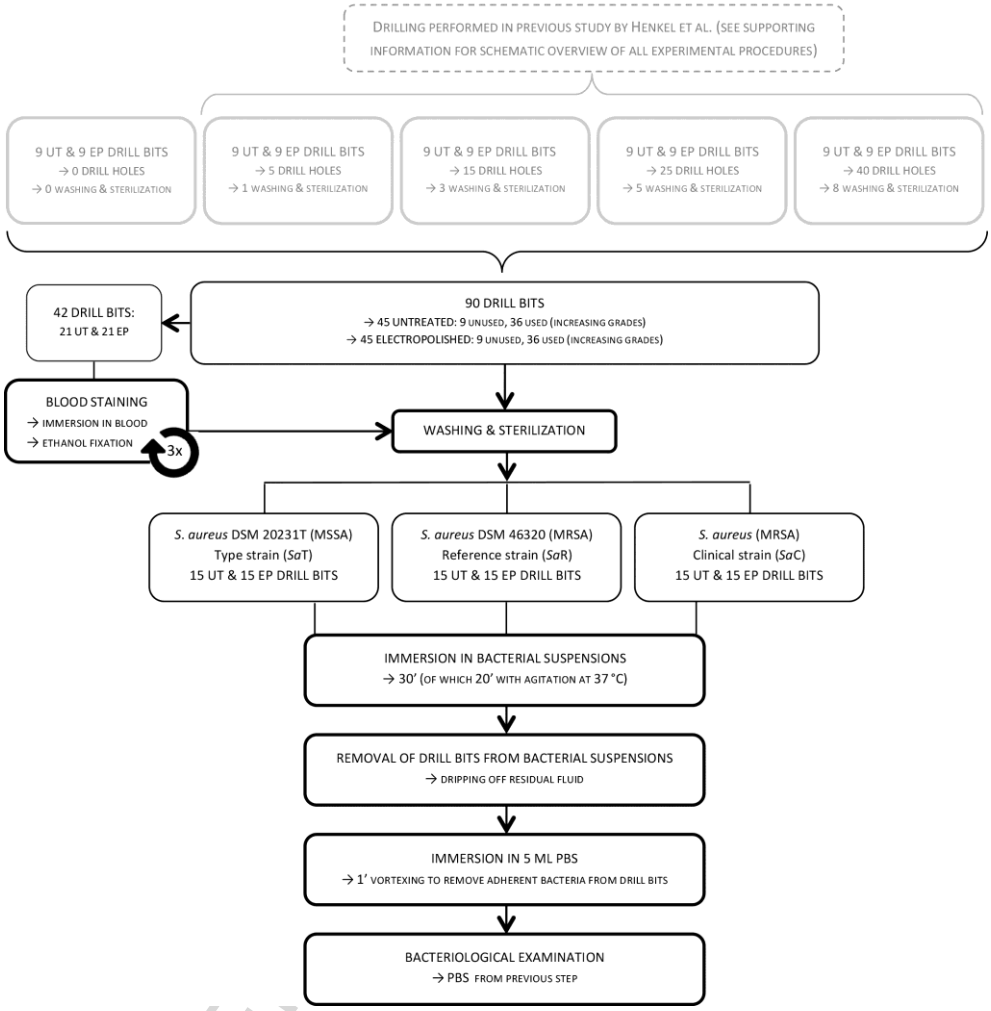

Figure 1

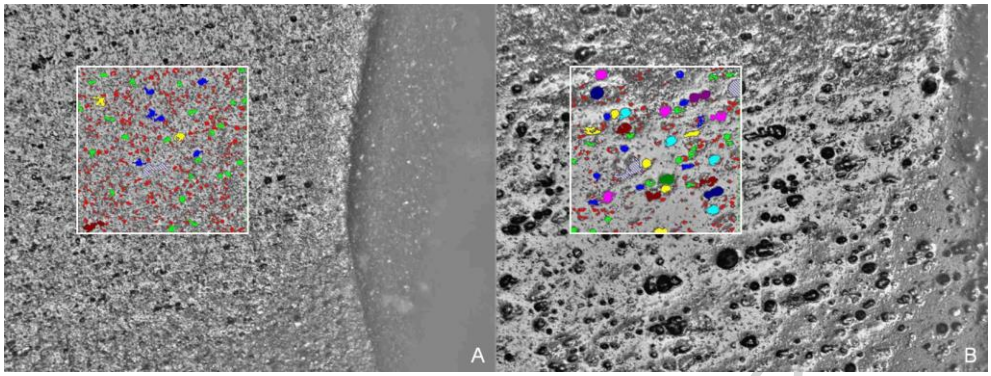

Figure 2

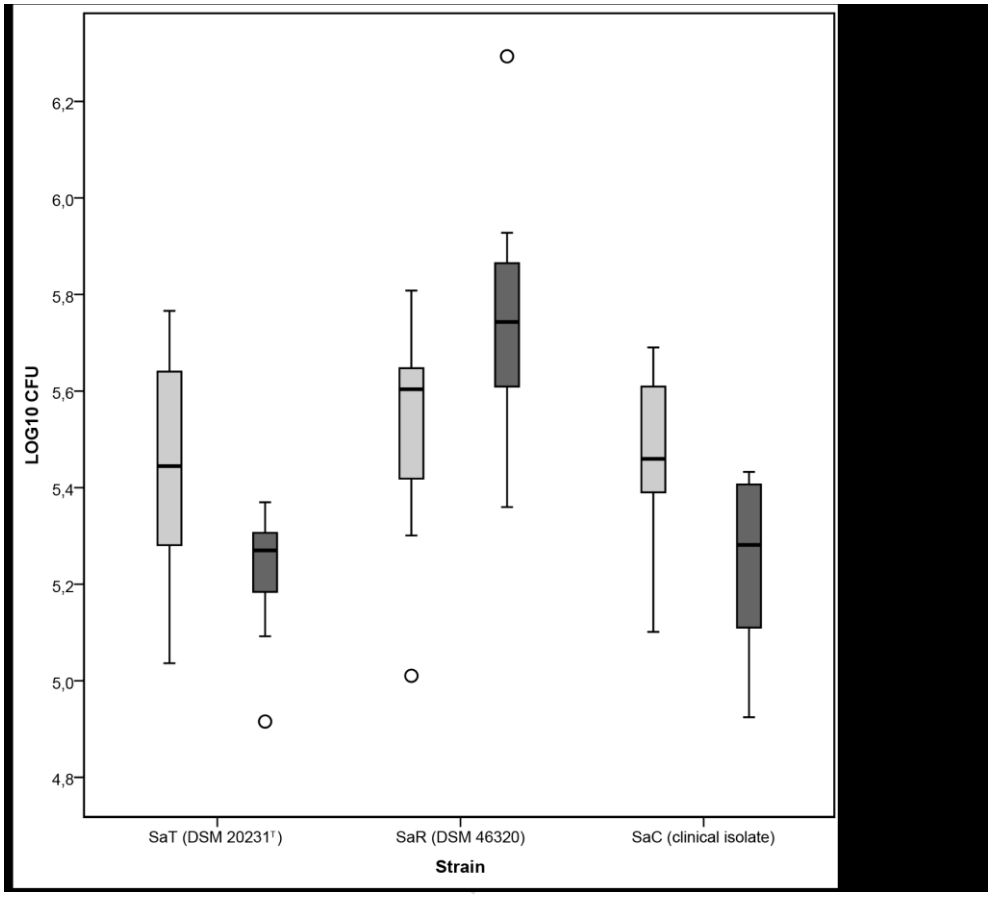

Figure 3

Highlights

- Drill bit contamination is an underinvestigated cause of surgical site infection.
- The surface of untreated and electropolished drill bits changes with use.
- Surface structures such as pores do not influence bacterial adhesion.
- Adherence of *S. aureus* on drill bits is strain dependent.
- Protecting drill bits from surface contamination should be surgical standard.